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CITATION:

SHIMOI, TOSHISHIGE. Studies on the Pathogenesis of Stress Ulcers. 日本外科宝函 1981, 50(2): 253-271

ISSUE DATE:

1981-03-01

URL:

http://hdl.handle.net/2433/208524

Arch Jpn Chir 50(2), 253～271, März, 1981

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# Studies on the Pathogenesis of Stress Ulcers

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Received for Publication, Dec. 21, 1980.

## Introduction

It has long been evident that acute ulcerations occur in the stomach and duodenum following various external injuries. In 1948 SELYE proposed the concept of an alarm reaction and diseases related to adaptation[31]. Gastric related ulcers follow a variety of stress conditions including multiple injuries and sepsis and acute gastro-doudenal ulcerations are known as "stress ulcers". Life in a complicated and industrialized society has resulted in an increase in the number of person with stress ulcers and improvements in the management of serious cases and the popularization of endoscopy can find much ulcers than before.

Numerous attempts have been made to produce stress ulcers experimentally in laboratory animals. Acute hemorrhagic erosion of the stomach can be produced simply and reliably by immersing rats in water, under conditions of restraint. Although this convenient model of stress ulcers does provide basic information concerning the pathogenesis of stress ulcers, the precise relationship between stress and the focal ulcer formation remains unknown. The rate of mortality in cases of icterus, shock, sepsis and uremia has reached 30%[5]. On the other hand, it is generally known that experimental stress ulcers occur even with a short time of stress and removal of the stress results in a rapid recovery. The acute occurrence and recovery of gastric lesions is attributed to microcirculatory changes in the gastric wall[11,13]. In recent years, it has become apparent that lysosomal enzymes act as a trigger in the process of decomposition of intra- and extracellular protein in mammals. The lysosomes are cell organelles which are limited by a membrane and contain a variety of hydrolytic enzymes that are activated at an acid pH. Cellular hypoxia produces an increase in the permeability of the lysosomal membrane and lysosomal enzymes are released into cells and surrounding extracellular spaces where they may cause damage[4]. Thus, continuous gastric mucosal microcirculatory disturbances may cause gastric mucosal ischemia and persistent ischemia may result in a release and activation of lysosomal enzymes.

To obtain information on the pathogenesis of stress ulcers, stress ulcers were produced by

Key words: Stress ulcer, Autonomic nerve, Gastric mucosal microcirculation, Lysosomal enzyme, Cathepsin.
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immersing rats in water under conditions of restraint and the influence of autonomic nerves and the changes in gastric secretion in the stressed rats were investigated. The microcirculatory changes in the gastric wall and the release of lysosomal enzyme, cathepsin, were observed histochemically and biochemically.

## Materials and Methods

### Experiment 1. Production of stress ulces

Male Wistar rats weighing from 250 g to 350 g were deprived of food for 24 hours, but were allowed free access to water. The rats were immobilized in each compartment of the stress cage designed by TAKAGI and OKABE,[35] then the cage was immersed vertically for 6 hours into a water bath of 23°C, to the height of the rat xyphoid process. The rats were then anesthetized with ether, the stomachs excised and opened along the greater curvature. The grossly visible ulcers were counted. More detailed measurements were not permitted because of the necessity to process the stomachs rapidly. The animals were weighed and sacrificed with an over dose of ether. Ulcerated areas were stained with Hematoxylin-Eosin and PAS.

### Experiment 2. Relation between the seasonal rhythm of the autonomic nervous tension and the stress ulcer formation

It was evident that only small hemorhagic erosions occurred with the same stress as described above during a period from July to August. Therefore, the incidence and the severity of stress ulcers in 60 rats during the four seasons were examined, and were expressed with the ulcer score reported by WILSON[41] (Table 1). In addition, eight patients with stress ulcers who were treated in our university hospital during the past 8 years were investigated.

### Experiment 3. Effect of vagotomy on stress ulcers

Truncal vagotomy and the HEINEKE-MIKULICZ type of pyloroplasty were performed on

**Table 1.** Classification and scoring of gastric ulcers produced by restraint

| Small ulcers (less than 0.5 mm in diameter) No. of ulcers | Intermediate ulcers (0.75～5 mm in diameter) No. of ulcers | Scores indicative of gastric damage |
|---|---|---|
| 5 | — | 0.25 |
| 10 | — | 0.50 |
| 15 | — | 0.75 |
| 20 | 1 | 1.00 |
| 25 | 2 | 1.25 |
| 30 | 3 | 1.50 |
| 35 | 4 | 1.75 |
| 40 | 5 | 2.00 |
| — | 6 | 2.25 |
| — | 7 | 2.50 |
| — | 8 | 2.75 |
| — | 9 | 3.00 |
| — | 10 | 3.25 |

60 rats. Thirty rats were given the same stress, described previously, 14 days after the T.V..

**Experiment 4. Effect of sympathectomy on stress ulcers**

The abdominal aorta was exposed, and the tissues between the level of the renal artery and celiac truncus around the aorta were removed in 7 rats. The rats were given the same stress as described previously, 4 days after the celiac ganglionectomy.

**Experiment 5. Estimation of the gastric secretion in stressed rats**

A tube to be connected to a bottle of saline was inserted subcutaneously through the back and advanced into the forestomach. Another tube through which the gastric juice and physiological saline solution were to be collected was also inserted subcutaneously through the back into the duodenum and the tip was left in the stomach. After the irrigation of the gastric cavity with 37°C physiological saline solution, the rats were placed face down on a board, the limbs fastened with threads, and were then immersed into the water bath for 6 hours after recovering from the anesthesia. Physiological saline solution was dropped into the stomach at the rate of 10 ml per hour. Every hour a sample was collected from the gastric fistulae and measured. Total acid output was expressed by the value obtained by titrating to pH 7.0 with 0.01 N NaOH according to the method of TÖPFFER MICHAELIS.

**Experiment 6. Observation of the microcirculation in the gastric mucosa**

a. Dry ice methanol wintergreen method[34] (DMW method)

A silk thread was tied around the abdominal aorta below the diaphragm and the abdominal aorta was cannulated with a polyethylene tube cranially, its tip placed just below the branching of the celiac truncus. As soon as the abdominal aorta was ligated with silk thread, 3 to 5 ml of India ink was injected into the aorta after perfusion of the stomach with physiological saline solution containing heparin (3000 unit in 1000 ml). The esophagus, stomach and duodenum were removed en masse, immediately frozen in methanol, cooled by dry ice, and then gradually over a 4 day period returned to room temperature. These organs were dipped into oil of winter-

**Table 2.** TAKAMATSU's staining method for cathepsin.

| | |
|---|---|
| ( 1) | Pieces of fresh tissues are frozen using acetone-dry ice. |
| ( 2) | Fix and dehydrate in acetone-alcohol solution at a temperature below 10°C for 48 hours. |
| ( 3) | Clear in xylene. |
| ( 4) | Embedded in soft paraffin at a temperature below 53°C and harden at 4°C. |
| ( 5) | Cut into 4~6 thick sections. |
| ( 6) | Attach section onto an object glass. |
| ( 7) | Dehydrate. |
| ( 8) | Remove paraffin in xylene. |
| ( 9) | Remove xylene in 99% alcohol. |
| (10) | Dehydrate. |
| (11) | Immerse for 24 hours in a prepared substrate mixture at 37°C. |
| (12) | Wash in water. |
| (13) | Dehydrate in air. |
| (14) | Clear in xylene. |
| (15) | Mount into balsam. |

green (Methyl salicylate) to be made transparent, carefully sectioned, and observed microscopically without staining.

b. FITC-Dextran method[20]

Three ml of 10 w/v% FITC-Dextran (Fluorescein isothiacyanate dextran, molecular weight approximately 39,000) was injected into the femoral vein and allowed to circulate throughout the body for 5 minutes. The stomach was removed, and the specimens of the gastric wall were rapidly frozen in acetone, cooled by dry ice and then freeze-dried for 5 days. These specimens were embedded in paraffin and cut into sections, approximately 10 $\mu$ in thickness. An olympus FLM fluorescein microscope with filters was used for the observations (excitation filter: $B_2$, Barrier filter: Y 52).

**Experiment 7. Relation between the lysosomal enzyme and stress ulcers**

a. Studies on the effect of predonisolone and prostaglandin $E_1$ on the occurrence of stress ulcers

Predonisolone (in doses of 20 mg/kg, 40 mg/kg, 80 mg/kg) was injected subcutaneously before the stress loading and also 30 minutes, 2 1/2 hours, 4 1/2 hours, after the loading. The stomachs were removed 6 hours later. The same procedure was used when $PGE_1$ in doses of 80 $\mu$g/kg, 160 $\mu$g/kg, 240 $\mu$g/kg were given.

b. Histochemical study of cathepsin in the gastric wall

The cathepsin staining was carried out by means of TAKAMATSU's method as shown in Table 2.[37,38]. Isolated stomachs were frozen in acetone cooled by dry ice, fixed, dehydrated at a temperature below 10°C, and embedded in soft paraffin with the melting point from 48°C to 52°C to protect cathepsin from decomposition. Substrate solution for the staining contained gelatin and 0.1% methylene-blue solution, as shown in Table 2. The tissue sections were immersed in the substrate solution for 24 hours, and observed microscopically.

c. Measurement of catheptic activities of the gastric mucosa

One hundred and thirty rats were separated into 6 groups: Group I. 25 normal rats, Group II. 25 rats given water immersion stress, Group III. 25 rats received T.V., Group IV. 25 rats given T.V. and stress, Group V. 15 rats given stress, and 80 mg/kg of predonisolone, Group VI. 15 rats given stress and 240 $\mu$g/kg of prostaglandin $E_1$. The catheptic activities were determined by UCHINO's method. The stomachs of five rats in each group were removed and frozen. The extracts of glandular stomachs were produced in an ice bath as shown in Table 3 and gelatin solution as substrate solution was added. This reaction mixture was incubated for 24 hours at 37°C. An increase of amino acids produced by degradation of glandular tissues due to the enzymatic reaction was measured by means of SOERENSEN's formol titration. The activities of cathepsin were indicated by the volume (ml) of 1/10 N NaOH solution used for titrating neutralization.

**Table 2.** Composition of substrate solution.

| | |
|---|---|
| Gelatine | 1 g |
| 0.1% methylene blue solution | 2 ml |
| McILVAINE's citrate buffer solution, pH 4.4 | 200 ml |

**Table 3.** Technical procedure of the determination of catheptic activity

1. Enzyme solution
   a) Gastric mucosa, 4 g (5 rats)
   b) Homogenize in a Waring blender after adding 3 volumes of glycerine water (1 : 1), keep cold, and mix with 1/6 volume of toluene……4 C, 24 hours
   c) Centrifuge at 3000 r.p.m. for 15 minutes and filter.
   d) The filtrate is then used as the test material.
2. Substrate solution: Pure gelatin, 4% buffer solution.
3. Buffer solution: McIlvaine's citrate buffer solution, pH 4.4.
4. Reaction mixtures:

| | Principal reaction mixture | | Contrasted reaction mixture | | | |
|---|---|---|---|---|---|---|
| | $I_0$ | $I_{24}$ | $II_0$ | $II_{24}$ | $III_0$ | $III_{24}$ |
| Enzyme solution | 2 | 2 | 2 | 2 | | |
| Glycerine water | | | | | 2 | 2 |
| Substrate solution | 10 | 10 | | | 10 | 10 |
| Buffer solution | 10 | 10 | 20 | 20 | 10 | 10 |
| Distilled water | 1 | 1 | 1 | 1 | 1 | 1 |
| (cysteine) | 1 | 1 | 1 | 1 | 1 | 1 |
| Toluene | 1 | 1 | 1 | 1 | 1 | 1 |

(Unit: ml)

5. Soerensen's formol titration.
   Enzyme Activity $=(I_{24}-I_0)-\{(II_{24}-II_0)+(III_{24}-III_0)\}$

## Results

### Experiment 1. Production of stress ulcers

Three hours of water immersion stress resulted in diffuse and oozing hemorrhages in the glandular portion of the stomach. Six hours of stress resulted in multiple hemorrhagic erosions which at a glance appeared to be uniform but were not so in size or shape, when closely observed; some were nearly circular while others were elongated (Fig. 1). Histologically, the ulcerated lesions formed the focal necroses of the upper half of the mucosa and were covered with blood

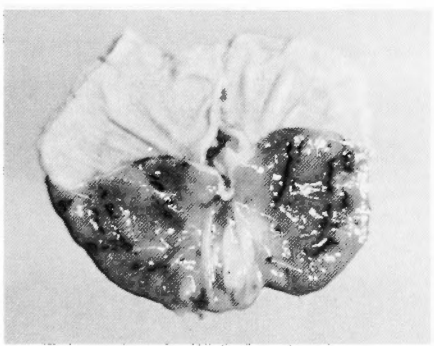


**Fig. 1.** Gross findings of the stomach of a stressed rat
Note the multiple irregular, serpentine, sharp hemorrhagic erosions.

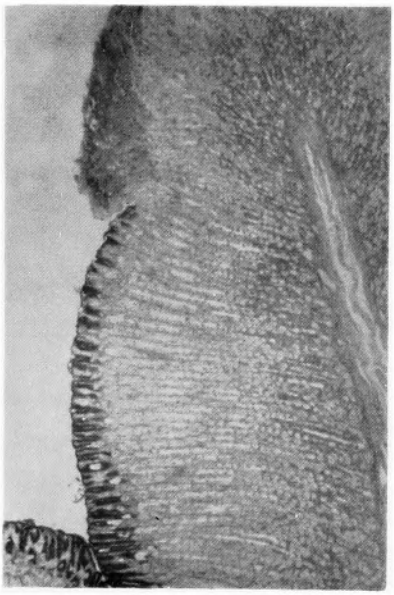


**Fig. 2.** Histological findings of the gastric corpus of a 6 hr stressed rat
Note the focal necrosis in the superficial half of the mucosa covered with blood clots, and the PAS reactive epithelium just adjacent to the hemorrhagic erosin. (H.E. & PAS. ×40)

clots, but the muscularis mucosae was not affected. The epithelium just adjacent to the hemorrhagic erosion was reactive in PAS staining (Fig. 2).

**Experiment 2. Relation between the seasonal rhythm of the autonomic nervous tension and stress ulcers**

The ulcer index of stress ulcers occurring in the summer was significantly lower as compared to indices of the other 3 seasons ($p<0.05$) (Table 4). Among the 8 clinical cases of stress ulcers, no occurrence was seen in May, June, July and August (Table 5).

**Experiment 3. Effect of vagotomy on stress ulcers**

Among 30 rats which were truncal vagotomized with the HEINEKE-MIKULICZ type of pyloroplasty and stressed, only 4 rats had fine hemorrhagic erosions (Fig. 3). The protective

**Table 4.** Seasonal ulcer score.

(60 rats)

| Score / Season | 2.00 | 2.25 | 2.50 | 2.75 | 3.00 | 3.25 |
|---|---|---|---|---|---|---|
| Winter | 0 | 1 | 5 | 4 | 3 | 2 |
| Spring | 0 | 3 | 5 | 6 | 1 | 0 |
| Summer | 4 | 6 | 3 | 2 | 0 | 0 |
| Autumn | 0 | 2 | 4 | 7 | 2 | 0 |

$\chi^2=30.174$ $p<0.05$

**Table 5.** Clinical cases of stress ulcers

| NO. | Patient Age, sex | Primary disease | Time of operation | Operation | Time of bleeding | Follow-up |
|---|---|---|---|---|---|---|
| 1 | M. T 86. M | rectal cancer | 9. 26 '70 | abdomino-perineal rectal amputation | 10. 26 '70 | died the following post-op. day |
| 2 | U. T 61. F | sigmoidal cancer | 1. 22 '73 | left hemicolectomy | 1. 23 '73 | total gastrectomy well |
| 3 | K. T 37. M | acute subdural hematoma | 2. 20 '74 | craniotomy | 3. 12 '74 | partial gastrectmy +SV···well |
| 4 | M. M 66. M | choledochal cancer | 3. 17 '74 | choledochojejuno-stomy | 4. 10 '74 | conservative died on 4.26 '74 |
| 5 | K. Y 64. M | cholelithiasis | 2. 7 '77 | cholecystectomy | 2. 14 '77 | conservative, well |
| 6 | T. K 27. M | duodenal ulcer | 3. 17 '77 | partial gastrectomy +SV | 3. 24 '77 | conservative, well |
| 7 | T. K 57. M | cholelithiasis | 2. 4 '78 | cholecystectomy | 2. 13 '78 | uremia died on 2.15 '78 |
| 8 | K. W 62. M | pancreatic cancer | 2. 8 '78 | endotoxin shock choledochoduo-denostomy | 12. 30 '77 | conservative died on 3.10 '78 |

effect of vagotomy on stress ulcers was evident at the rate of 87 percent

**Experiment 4. Effect sympathectomy on stress ulcers**

Among 7 rats which were celiac ganglionectomized and stressed, all had hemorrhagic erosions, but these lesions were not so large as those in the control group (Fig. 4). The protective effect of sympathectomy on stress ulcers was 50 percent, according to the ulcer index.

**Experiment 5. Estimation of gastric secretion in stressed rats**

The gastric acid output per one hour period in rats immersed and restrained in water gradually decreased for the initial 4 collection periods, and gradually increased for the final 2 collection periods (Fig. 5, Table 6). Although there were individual differences in the gastric acid output in the stressed rats, in each of the rats there was a decrease in the gastric acid output for 3 to 4 hours just after the stress.

**Experiment 6. Observation of the microcirculation in the gastric mucosa**

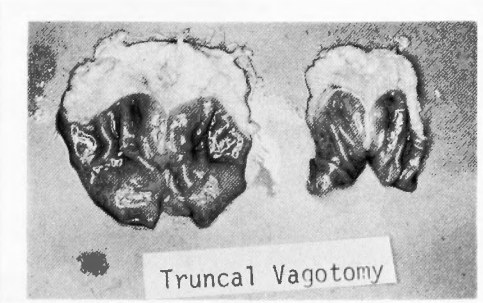



**Fig. 3.** The stomach of a bilateral truncal vagotomized, 6 hr stressed rat
Note the lack of erosin (left) and a few hemorrhagic erosions (right).

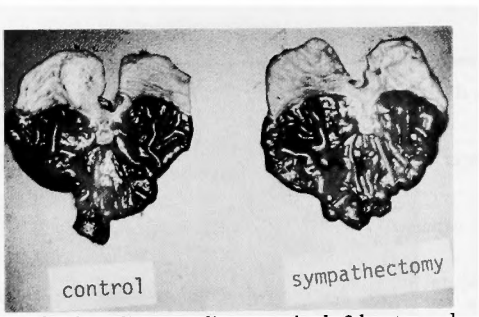



**Fig. 4.** The stomach of a celiac ganglionectomized, 6 hr stressed rat
Note the fewer and smaller hemorrhagic erosions, as compared with the control stomach.

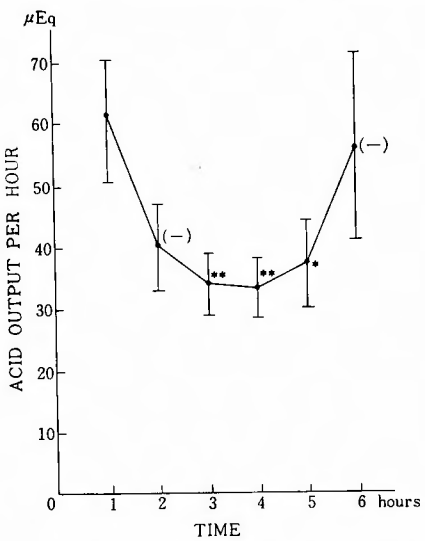



**Fig. 5.** The gastric acid output in stressed rats with gastric fistula
Note the gradual decrease up to 4 hours in restraint + cold stressed rats.
Statistically significant against first collection (Student's *t*-test)
**P<0.05, *p<0.10, (−) P>0.10.

**Table 6.** Acid output in rats with gastric fistulae
(cold+restraint)

| N \ T | 1 | 2 | 3 | 4 | 5 | 6 hours |
|---|---|---|---|---|---|---|
| 1 | 26 | 23 | 20 | 23 | 24 | 29 |
| 2 | 81 | 45 | 38 | 26 | 25 | — |
| 3 | 90 | 68 | 38 | 28 | 29 | 35 |
| 4 | 54 | 40 | 39 | 55 | 64 | 88 |
| 5 | 85 | 23 | 13 | 21 | 11 | 20 |
| 6 | 41 | 27 | 30 | 31 | 46 | 61 |
| $\bar{x}$ | 62.8 | 40.9 | 34.0 | 33.7 | 37.0 | 57.8 |
| SD | 26.3 | 18.0 | 15.1 | 13.9 | 19.9 | 37.0 |
| SE | 10.7 | 5.8 | 5.7 | 5.2 | 7.5 | 15.1 |

(Unit: μEq)

**Fig. 6.** Microangiograms of the gastric mucosa in a normal rat
A. Transverse section of the gastric wall taken with DMW method (×100)

a. DMW method

Submucosal arteries give off numerous arterioles which penetrate the muscularis mucosae and immediately branch into capillaries at the base of the gastric glands in normal rats. The mucosal capillaries enclose individual gastric glands with their rich networks, run upward and connect with the collecting veins. Collecting venules penetrate the mucosa in a straight line, penetrate the muscularis mucosae and are drained finally by submucosal veins (Fig. 6. A). Microcirculation of the gastric mucosa after 3 hours of stress is shown in Fig. 7. The mucosal capillaries were narrower in the lower layer of the mucosa. Vascular engorgement was demonstrated in the upper layer of the mucosa. When stressed for 6 hours, the gastric mucosa showed vascular engorgement and hemorrhagic erosions in the upper layer of the mucosa. Fewer, narrower and more irregular capillaries were found at the base of the erosions (Fig. 8. A).

b. FITC-Dextran method

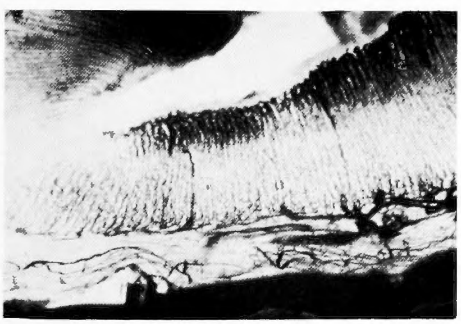
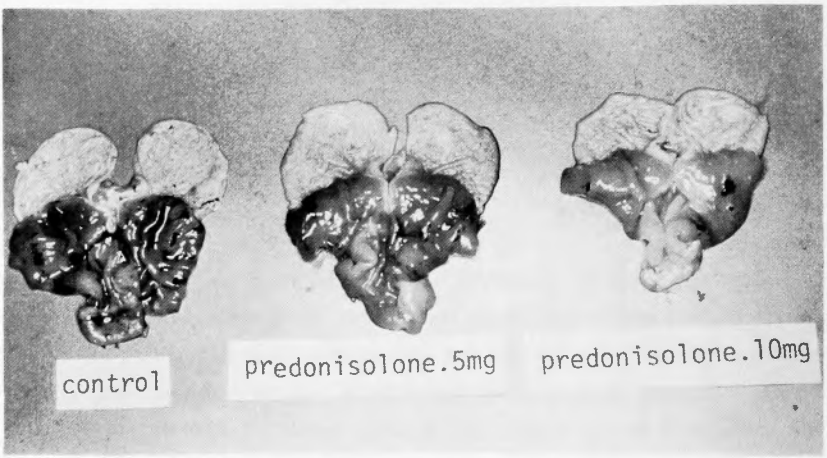
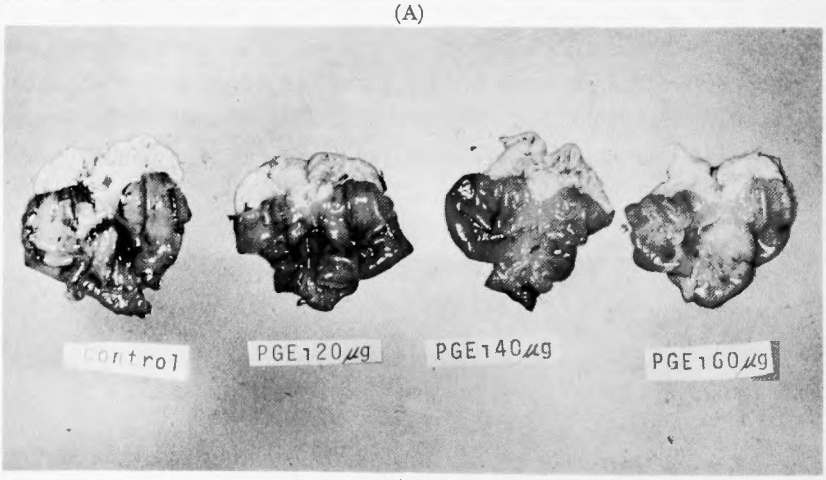


**Fig. 7.** Microangiograms of the gastric mucosa in a 3 hr stressed rat
Note the congestion at the surface and the narrowing capillaries at the deep mucosa. (×40)

**Fig. 8.** Microangiograms of the gastric mucosa of a 6 hr stressed rat
A. DMW method (×40)




(A)




(B)

**Fig. 9.** Gross findings of the stomach in the stressed rats administered with lysosomal stabilizers
A. Administration of predonisolone
B. Administration of prostaglandin $E_1$
Note the changes of mucosal lesions in proportion to the dosage of the lysosomal stabilizers.

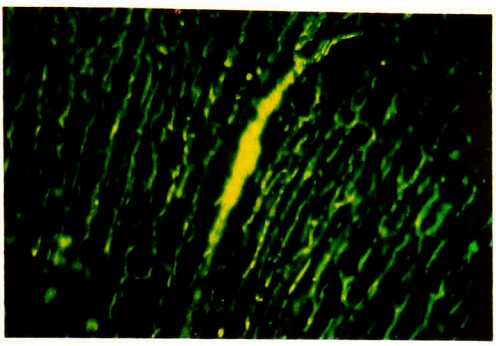


**Fig. 6.** Microangiograms of the gastric mucosa in a normal rats
B. FITC-Dextran method
Note the spinning rich capillaries networks and the collecting venules receiving capillaries at the surface. (×100/1.3)

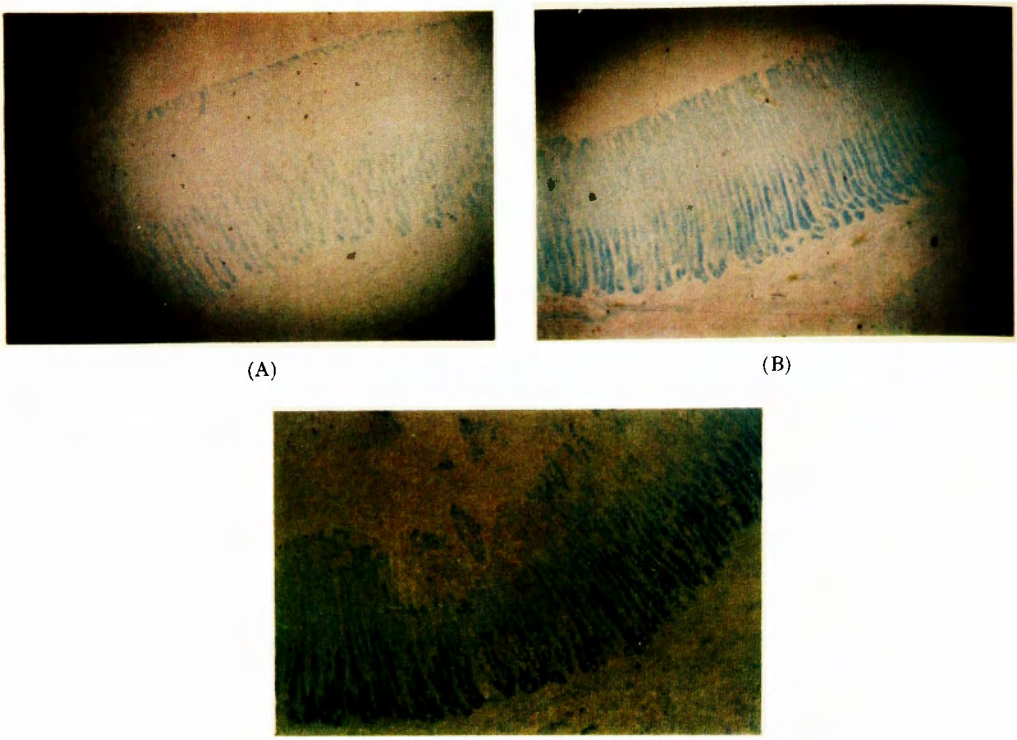
The findings of these specimens were similar to those in the case of the DMW method. In normal gastric mucosa, the mucosal capillaries enclosed individual gastric glands by their rich networks and ran upward to connect with the collecting veins (Fig. 6. B). When stressed for 6 hours, the gastric mucosa showed hemorrhagic erosions, and spillage of FITC-Dextran was evident in the upper layer of the mucosa (Fig. 8. B.).

**Experiment 7. Relation between the lysosomal enzyme and stress ulcers**

a. Studies on the effect of predonisolone and prostaglandin $E_1$ on the occurrence of stress ulcers

Predonisolone and prostaglandin $E_1$ had dose-related protective effects on the stress ulcer formation (Fig. 9. A. B). Total prevention of stress ulcers occurred with the administration at

**Fig. 8.** Microangiograms of the gastric mucosa of a 6 hr stressed rats
B. FITC-Dexxtran method
Note the hemorrhagic erosion at the surface and the narrowing, fewer, irregular capillaries at the base of the erosion. (×100/1.3)



(A) (B)

(C)

**Fig. 10.** Histochemical findings of cathepsin in the gastric wall of the rats
A. Normal rat: Note the cathepsin staining at the surface and at the deep mucosa. (×40)
B. A 3 hr-stressed rat: Note the cathepsin staining throughout the mucosa. (×40)
C. A 6 hr-stressed rat: Note the strong cathepsin staining just adjacent to the erosin. (×40)

a dose of 80 mg/kg of predonisolone and of 240 $\mu$g/kg of prostaglandin $E_1$.

b. Histochemical study of cathepsin in the gastric wall

In the normal gastric wall, only the superficial and deep portions of the mucosa were stained (Fig. 10. A). When stressed for 4 hours, cathepsin staining was seen throughout the mucosa

**Table 7.** Catheptic activities of gastric mucosa of rats.

(1/10 N NaOH ml)

| NO. | Control | Stress | T.V | T.V+stress | P+stress | PGE.+stress |
|---|---|---|---|---|---|---|
| 1 | 0.11 | 0.25 | 0.17 | 0.19 | 0.17 | 0.18 |
| 2 | 0.12 | 0.27 | 0.15 | 0.19 | 0.19 | 0.23 |
| 3 | 0.16 | 0.31 | 0.17 | 0.24 | 0.25 | 0.27 |
| 4 | 0.18 | 0.32 | 0.20 | 0.25 | | |
| 5 | 0.20 | 0.33 | 0.24 | 0.26 | | |
| $\bar{x}$ | 0.154 | 0.276 | 0.186 | 0.226 | 0.203 | 0.227 |
| SD | 0.038 | 0.050 | 0.035 | 0.033 | 0.042 | 0.045 |
| SE | 0.017 | 0.022 | 0.015 | 0.015 | 0.024 | 0.026 |

of the gastric wall (Fig. 10. B). When stressed for 6 hours, the superficial necrotic region of the gastric wall was little stained, while the surrounding region was well stained (Fig. 10. C).

c. Measurement of catheptic activities of the gastric mucosa

The catheptic activities of the glandular mucosa of the stomach in 6 groups are shown in Table 7. The mean value in each group was as follows: in Group I, $0.15\pm0.017$, in Group II, $0.27\pm0.022$, in Group III, $0.18\pm0.015$, in Group IV, $0.22\pm0.015$, in Group V, $0.20\pm0.024$ and in Group VI, $0.22\pm0.026$. The stomachs of the rats given stress (Group II) showed a higher level of catheptic activities than did the normal rats (Group I). The stomachs of rats given stress plus vagotomy (Group IV), predonisolone (Group V), or $PGE_1$ (Group VI), respectively, showed a lower level of catheptic activities than those of rats given stress alone (Group II).

## Discussion

In 1936[32], SELYE reported that systemic changes in many organs including gastric ulcers sometimes occurred after inflicting stress on rats, irrespective of the type of stress. Since then experimental gastric ulcers have been produced by inflicting a variety of stress on various species. OKABE[29] reported that water-immersion and restraint related stress had resulted in the production of gastric ulcerations in a number of rats with the same degree of incidence and severity. This method of water immersion and restraint stress has been used as a model of stress ulcers by many investigators. WILSON[41] reported the seasonal and monthly variations in the severity and incidence of stress-induced ulcers in rats indicating a cyclic variation with maximum incidence in December and minimum incidence in June. In the present work, the ulcer index of stress ulcers in the summer was significantly lower than that in other seasons. In the clinical cases of stress ulcers, no occurrence was seen in May, June, July and August. MATSUO and SEKI[17] reported that cervical sympathectomy was significantly effective on the speed of healing of tissue defects in rabbit ears in the winter, but was not effective in the summer. He suggested that the difference in the autonomic nervous tension between in the winter and in the summer might account for these results.

There have been numerous reports concerning mechanisms related to the transmission of stress to the stomach. FRENCH[7,8] et al classified the stress ulcers into the ulcerative and the hemorrhagic types and reported that destructive lesions of the anterior hypothalamus had produced hemorrhagic erosions (protected by sympathectomy) and those of the posterior parts of hypothalamus crater formation (protected by vagotomy). MATSUO[18] observed that stimulation of the posterior area of the orbital surface of the frontal lobe produced an exacerbation of the gastric motility and the stasis of the gastric blood flow, while stimulation of the same area in vagotomized cats produced an inhibition in gastric motility. He found that the same procedure in splanchinectomized cats produced an exacerbation of the gastric motility and no change of the gastric blood flow. He explained that the stimulation of the posterior area of the orbital surface of the frontal lobe passed to the stomach by way of the vagus produced an exacerbation of the gastric motility and by way of sympathetic nerves produced a constriction of the gastric vessels. He[19] added the data obtained by GRAY[10] and FRENCH[7,8] to his results of investigation and a

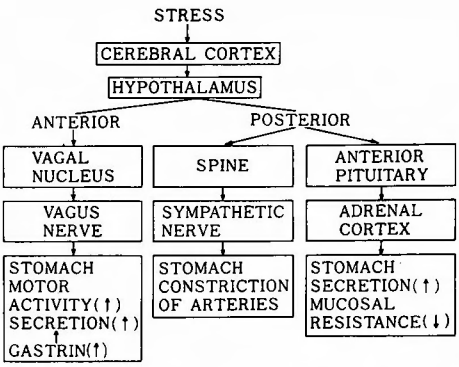



**Fig. 11.** Possible mechanisms of transmission of stress to the stomach

summary is shown in Fig. 11.

Different forms of stress stimulate the cerebral cortex and are transmitted to the stomach by way of the anterior hypothalamus, the parasympathetic nerve, and by way of the posterior hypothalamus, the sympathetic nerve, the pituitary and the adrenal glands. The related reports include 'mucosal energy metabolism' by MENGUY and MASTERS[21], 'microcirculatory and mast cell changes' by GUTH and HALL[11], 'changes of protective mechanism of the gastric mucosa' by KIRA[16], 'back diffusion theory' by DAVENPORT[3], 'barrier disruption of various pathogenesis' by SKILLMANN and SILEN[33], relation of infection and sepsis by GOLDMANN and ROSOFF[9].

The protective effect of vagotomy on stress ulcers is well known[24], but the effect of sympathectomy is not well understood. In the present work, truncal vagotomy prevented ulcer formation at the rate of 87%, therefore, the protective effect of vagotomy on stress ulcers was apparent. MOCHIZUKI[23] reported that the protective effect of sympathectomy on stress ulcers was obtained at the rate of 50%. In the experiments reported in this paper, however, stress ulcers occurred in all of the sympathectomized and stressed animals, despite the fact that the severity of ulcers was not so great as in the control animals. Sympathectomy had a protective effect on stress ulcers at the rate of 50% in the ulcer index, but a significant protective effect was not recognized. MURYOBAYASHI[25], et al reported that in the cervical vagus nerve and its gastric branch in cats and dogs, green fluorescent materials representing cathecholamines were seen along the myelinated fibers and that the ligation of the nerve resulted in an intensification of the fluorescence above the ligature. He also reported that the vagus nerve of cats and dogs contained adrenergic nerve fibers, which originated from or passed through the superior cervical ganglion. OHSUMI[28] observed that the cardiac region of the stomach received the adrenergic nerve fibers from the vagus nerve and from the sympathetic celiac ganglion. Therefore, the sympathetic stimuli are probably transmitted to the stomach, even when celiac ganglionectomy has been performed.

There has been no agreement as to the gastric secretory changes in stress ulcers. MENGUY[22] and BRODIE[2], et al demonstrated the decrease of gastric secretions in pylorus ligated and restrained rats. HASE[12], et al studied the gastric secretion (secretory volume, acid concentration, total gastric acid output) in the pylorus ligated and rotationally stressed rats and found that the gastric

secretion was highest in stressed rats which did not develop ulcers, intermediate in stressed rats which developed ulcers, and lowest in the control group. O'NEILL[30], et al found no increase in the gastric acid output in the burned patients complicated with curling ulcers, however he did find that the gastric acid output of the burned patients who developed ulcers showed normal values, whereas the patients who developed no ulcers showed lower values than the normal. In the present work, the gastric acid output gradually decreased for some hours after the infliction of stress (Fig. 5, Table 6).

HAYASHI[14] reported that an increase of the gastric acid output might not be important in stress ulcer formation and that even a small quantity of gastric acid might play a significant role in stress ulcer formation in the stomach in which the protective mechanism against ulceration was weakened under stress.

HASE and MOSS[13] reported that, under stress, mucosal ischemia was induced by contraction of the connecting arterioles and that persistent focal ischemia of the gastric mucosa triggered tissue damage and development of stress ulcers.

GOLDMANN and ROSOFF[9] reported that the mucosal ischemia might result in part from the muscular contraction and extrinsic compression of the intramural vessels and that this would lead to degeneration of the superficial mucous cells. He also reported that the ulceration resulted from the action of the luminal acid on the mucosa that had been previously damaged by ischemia.

GUTH and HALL[11] observed the mast cell degranulation and the vascular engorgement in the mucosa just below the surface epithelium in restrained rats. They reported that the hyperemic region of the mucosa was susceptible to damage by acid, following the formation of stress ulcer. In rats that were stressed for 3 hours the author found that the microcirculation of the mucosa showed engorgement in the upper layer of the mucosa and narrowed capillaries in the deep layer of the mucosa (Fig. 7). In rats stressed for 6 hours, hemorrhagic erosion was demonstrated with engorgement in the upper layer and ischemia in the deep layer of the mucosa (Fig. 8).

JANOFF[15] reported that shock induced by trauma, ischemia and bacterial endotoxin was consistently associated with an increase in blood level of acid phosphatase and $\beta$-glucuronidase. BITENSKY[1] studied histochemically the spleen of rabbits that received shock induced by bleeding and observed the release of lysosomal enzymes into the cells. WEISSMANN, et al reported that a significant increase in the release of cathepsin and $\beta$-glucuronidase was evident in the large fraction of homogenized liver in endotoxin shocked rabbits, and that a reduction in these levels was observed in endotoxin shocked rabbits which had been given hydrocortisone[39,40]. FERGUSON[6], et al produced serotonin-induced gastric ulcerations in rats and found an increase in the activities of cathepsin D in the serum. They observed the protective effect of prostaglandin $E_1$ on ulcer formation and the release of cathepsin D. In the present work, the apparent protective effect of predonisolone and $PGE_1$ on the ulcer formation in stressed rats was dose dependent (Fig. 9). These results show the protective effect of lysosomal stabilizers (predonisolone, $PGE_1$) on stress ulcer formation and suggest that stress ulcer formation may be closely related to the activation of lysosomal enzymes.

Cathepsin is an auto-tissue protein splitting enzyme discovered by SALKOWSKI in 1890. There are 5 isomers of cathepsin, A, B, C, D and E, due to SH-dependency, optimum pH, and substrate specificity. It is considered that cathepsin B, D and E function as an endopeptidase and that cathepsin C functions as an exopeptidase. Cathepsin D plays a significant role in the initial stage of protein decomposition and acts on many substrates including hemoglobin, gelatin and casein[27]. In experiments herein, the reaction mixture was adjusted pH 4.5 using gelatin as a substrate. Cathepsin C and D were assayed.

In the histochemical study of the catheptic activities, the TAKAMATSU stain method[37,38] was used and gelatin and methylene blue solution were used as substrates.

When gelatin and methylene blue solution come into contact with tissues, the gelatin is decomposed into lower molecules by the proteolytic action of cathepsin. At that point, methylene blue combining with the decomposed gelatin becomes isolated and is fixed to the tissue in which cathepsin is activated. In the experiments herein, the gastric wall in stressed rats was stained strongly positive for cathepsin, on the surface and well as in the depth of the mucosa, as compared with the normal gastric wall. The necrotic lesion in the superficial region of the mucosa was little stained, but the surrounding area was stained remarkably. These findings suggest that cathepsin is activated in the region where microcirculatory disturbance has occurred and that the activated cathepsin causes a decomposition of auto-tissue protein of the mucosa and leads to erosion. NISHIBORI[26] observed histochemically the activation of acid phosphatase and $\beta$-glucuronidase in the subcutaneous tissue of the posterior limb in rats 16 hours after the ligation of the bilateral common iliac arteries and veins, and the bilateral femoral arteries and viens. He also observed that the stains disappeared during the advanced stage of necrosis. TAKAHASHI[36] reported that the catheptic activities in the intact part of the gastric wall in the gastric ulcer patients were specifically enhanced in comparison with duodenal ulcers or gastric cancers and that there were significantly higher activities of cathepsin in the gastric wall in the histamine-induced gastric ulcer group than in the non-ulcer or normal group in rats. The author found that the catheptic activities of the gastric mucosa in stressed rats were significantly enhanced as compared with normal gastric mucosa. However, the increase of catheptic activities of the gastric mucosa was inhibited significantly by truncal vagotomy and administration of predonisolone or prostaglandin $E_1$. These findings suggested that the activities of the auto-tissue protein splitting enzyme, one of the lysosomal enzymes, cathepsin, might play an important role in the pathogenesis of stress ulcer formation.

## Conclusion

To obtain additional information on the pathogenesis of stress ulcers, the autonomic nervous system, gastric secretion, gastric microcirculation and lysosomal enzymes were studied in water-immersed and restrained rats. The following results and conclusions were obtained:

1) In the 3 hour-stressed rats, diffuse and oozing hemorrhages were seen in the stomach. In the 6 hour-stressed rats, multiple hemorrhagic erosions were evident in the glandular portion.

2) A seasonal rhythm in the incidence and the severity of stress ulcers was recognized ex-

perimentally, and in clinical cases, the incidence was low in the summer months.

3) The protective effect of truncal vagotomy on stress ulcers was seen at the rate of 87%.

4) The protective effect of sympathectomy on stress ulcers was seen at the rate of 50%.

5) The gastric acid output in stressed rats with gastric fistulae gradually decreased for a few hours after the infliction of stress, then increased.

6) Regarding gastric mucosal microcirculation, congestion in the upper layer of the mucosa and ischemia in the deep mucosa were seen 3 hours after stress. Hemorrhagic erosion in the superficial mucosa, and constriction and breakage of capillaries at the base of the erosion were seen in the 6 hour-stressed rats.

7) The lysosomal stabilizers (predonisolone, prostaglandin $E_1$) had dose dependent protective effects on stress ulcer formation.

8) In the histochemical investigation of cathepsin using the TAKAMATSU stain method, the activities of cathepsin increased in the gastric mucosa, particularly in the surrounding area of the necrotic region in stressed rats.

9) The catheptic activities of the gastric mucosa in stressed rats were significantly enhanced as compared with findings in the normal gastric mucosa, but the increase of the catheptic activities was inhibited by truncal vagotomy and or administration of predonisolone or prostaglandin $E_1$.

All these findings suggest that the stress causes the microcirculatory disturbances in the gastric mucosa by way of the autonomic nerve, and that with the digestion and erosion of the mucosa, "stress ulcers" are produced by activated cathepsin, pepsin and acid on the mucosa when the tissue resistance is poor.

Vagotomy, predonisolone and prostaglandin $E_1$ all have protective effects on stress ulcer formation through suppression of the activities of the lysosomal enzyme, cathepsin.

**Acknowledgements**

Sincere gratitude is extended to Prof. KOICHI ISHIGAMI for his guidance and pertinent suggestions throughout this study.

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## 和文抄録

# ストレス潰瘍の成因に関する研究

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ストレス潰瘍の成因に関する臨床的および実験的研究報告は数多くみられるが，その詳細な発生機序はいまだ充分に解明されていない．そこでラットに水浸拘束を負荷してストレス潰瘍胃を作成し，胃粘膜微小循環および胃粘膜 lysosome 酵素 cathepsin の活性を中心に検討して，次のような成績を得た．

1) ラットに水浸拘束を負荷すると，腺胃部に3時間後にはびまん性出血を，また6時間後には限局性の出血壊死が多発することを認めた．

2) 年間を通じてストレス潰瘍を作成し，その潰瘍の数，大きさを潰瘍係数で現わした．さらに臨床例についても検討したところ，ストレス潰瘍の発生には年間リズムがあり，夏期には発生しにくいことが示された．

3) 幹性迷切＋幽門形成術を施行し，2週後にストレスを負荷すると，87%の潰瘍発生の抑制を認めた．一方腹腔神経節摘除術を施行し，4日後にストレスを負荷すると，50%の潰瘍係数の低下を認めた．

4) 胃瘻を作成したラットにストレスを負荷すると，胃酸分泌量は水浸拘束群では4時間後まで漸減し，のちには漸増した．

5) 胃粘膜微小循環を瞬間凍結墨汁法および色素注入法（FITC-dextran 法）による螢光組織化学的方法によって観察した．ストレス負荷後3時間より粘膜表層のうっ血像および深層の血管収縮像を認め，6時間後には粘膜表層のうっ血像，出血およびびらん像，深層の血管収縮像，びらん像直下の血管数の減少および血管の断裂像を認めた．

6) lysosome 膜安定化物質である predonisolone (20 mg/kg, 40 mg/kg, 80 mg/kg) または prostaglandin $E_1$ (80 μg/kg, 160 μg/kg, 240 μg/kg) を投与することによって，ストレス潰瘍の発生はその投与量に比例して抑制された．

7) ゲラチン，メチレンブルーを基質とする高松らの組織化学法によって検討したところ，ストレス潰瘍胃壁においては cathepsin の活性が亢進していることを認めた．

8) 腺胃部胃粘膜の cathepsin 活性をゲラチンを基質として Soerensen のフォルモール滴定法によって測定し，中和滴定に要した N/10 NaOH 量 (ml) で示した．正常胃は 0.15±0.017，ストレス潰瘍胃は 0.27±0.022，幹性迷切胃は 0.18±0.015，幹性迷切・ストレス負荷胃は 0.22±0.015，predonisolone 80 mg/kg・ストレス負荷胃は 0.22±0.024，$PGE_1$ 240 μg/kg・ストレス負荷胃は 0.22±0.026 の値を示し，ストレス潰瘍胃粘膜では正常胃に比して活性の亢進を認めた．一方迷切の施行や predonisolone, または $PGE_1$ の投与によってその活性の亢進は軽度となった．

以上の実験成績より，ラットに水浸拘束を負荷すると，ストレスは自律神経を介して胃粘膜の血行障害をひき起こし，胃粘膜抵抗減弱部位に活性化された cathepsin および酸・ペプシンの作用が加わり，粘膜の消化，びらんが起こり，潰瘍を発生せしめること，さらに迷切，predonisolone または $PGE_1$ などは lysosome 酵素カテプシンの活性の抑圧を介してストレス潰瘍の発生を抑制することが明らかとなった．